

The Formation of Benzyl Thiocyanate in the Seeds of *Lepidium sativum*

In order to find an explanation for the observation of Gmelin and Virtanen¹ that large amounts of benzyl thiocyanate (BTC) are formed in addition to benzyl isothiocyanate (BITC) in crushed, moistened seeds of *Lepidium sativum*, we have studied the remarkable reaction more thoroughly. At first we found that the ratio of BTC to BITC, which is about 2.5 at 23°C when water is added to the crushed seeds, is reversed to about 0.3 when 0.5 M citrate buffer (pH 5.2) is added instead of water. The pH of the solution has no greater effect on the ratio of the reaction products in the range 4.6–6.6. Neutral salts (NaCl and KCl) have a similar effect as the citrate buffer (Table 1).

Table 1. Formation of benzyl thiocyanate (BTC) and benzyl isothiocyanate (BITC) in crushed *Lepidium sativum* seeds when water or different buffer and salt solutions were added to the crushed material, γ /100 mg of seeds. Temperature \sim 22°C, time 15 min., pH 5–5.2.

Additions	BTC	BITC	BTC + BITC	$\frac{\text{BTC}}{\text{BITC}}$
Water	728	310	1038	2.35
0.3 M citrate buffer	268	806	1078	0.33
0.5 " " "	224	814	1038	0.25
1.0 " " "	216	799	1015	0.27
0.1 " acetate "	564	446	1010	1.27
0.5 " " "	436	595	1034	0.73
1.75 % NaCl	568	396	964	1.43
5.80 " "	252	432	684	0.58
16.80 " "	140	396	536	0.35
22.70 " "	80	360	420	0.22
2.24 " KCl	428	461	889	0.93
7.50 " "	300	403	703	0.74
22.70 " "	88	418	506	0.21
14.20 " Na ₂ SO ₄	444	274	718	1.62
Water, heated seeds*	0	1360		

* Seeds were heated at 100°C for 5 h to destroy the enzymes. A raw »myrosinase« preparation from *Lepidium sativum* was added to the inactivated, crushed material.

The total amount of BTC and BITC was about the same irrespective of variations in their ratio. This is decisive proof of the formation of BTC and BITC from the same thioglucoside, glucotropaeolin, in *Lepidium* seeds. Only BITC was formed from the glucotropaeolin in the crushed seeds of *Tropaeolum maius*.

Temperature also has a strong effect on the ratio of BTC to BITC (Fig. 1).

The results were not easy to understand. We first believed that glucotropaeolin in *Lepidium* seeds is bound to a basic group of some protein and that rearrangement occurs on the sulfur atom after the splitting of glucose. We then began to study the rate of the reaction and the influence of temperature on the reaction products. The reaction rate is extremely high, for the reaction goes to completion in a few minutes, even at 2°C (Table 2).

From the results in Table 2 it appears that after a few seconds at 2°C the ratio of BTC to BITC is < 0.3 , but that it rises very rapidly. After 60 seconds it is already 2.7 and after 5 min., when the reaction is completed, 4.7. The results suggest that the Lossen rearrangement after the enzymic splitting of glucose and sulfate leads to the formation of BITC also in crushed and moistened *Lepidium* seeds. The BITC is, however, rapidly isomerized to BTC. Because BITC is not spontaneously isomerized to BTC — e.g. in crushed, moistened *Tropaeolum* seeds only BITC is formed from glucotropaeolin — the

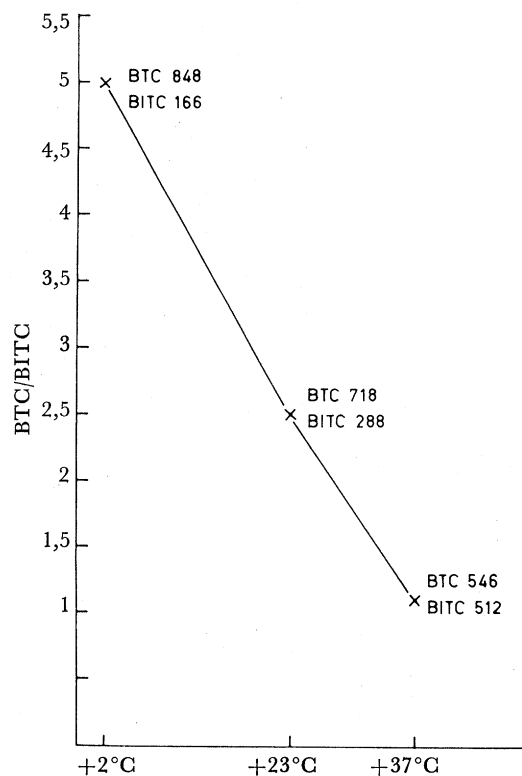


Fig. 1. The dependence of the formation of benzyl thiocyanate and benzyl isothiocyanate in crushed, moistened seeds of *Lepidium sativum* on temperature. Time 5 min.

Table 2. The formation of BTC and BITC (γ/100 mg seeds) when the enzymic reaction was terminated after very short reaction times after the addition of water to the crushed seeds of *Lepidium sativum*. Temperature 2°C.

Reaction time, sec.	BTC	BITC	BTC + BITC	BTC/BITC
10—15*	188	623	811	0.30
30*	456	392	848	1.16
60	672	247	919	2.72
120	736	234	970	3.14
180	800	187	987	4.28
300	848	181	1029	4.69
1800	849	173	1022	4.91

*The reaction times under 60 seconds are only approximate.

reaction is obviously enzymic. This means that *Lepidium* seeds should contain an isothiocyanate isomerase, an until now unknown enzyme. The probable pathway for the formation of benzyl thiocyanate (III) from glucotropaeolin (I) over benzyl isothiocyanate (II) is that shown in Fig. 2.

On the basis of the values of the ratio of BTC to BITC at different temperatures, the activity of the isomerase decreases when the temperature rises from 2 to 37°C (Fig. 1). The enzyme is not present in the water extract prepared from *Lepidium* seeds, because, as mentioned above, the addition of this extract to heated, crushed *Lepidium* seeds leads only to the formation of BITC. The investigation on the isomerase and the factors which influence the formation of thiocyanic esters in plants is in progress in this laboratory.

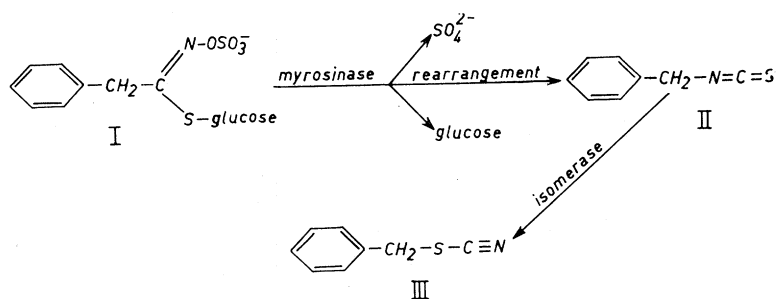


Fig. 2. The formation of benzyl isothiocyanate (II) and benzyl thiocyanate (III) from glucotropaeolin (I)

A detailed report, in which also the method we have developed for the determination of benzyl thiocyanate is described, will be published in *Acta Chemica Scandinavica*.

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Reference

1. Gmelin, R. and Virtanen, A. I. *Acta Chem. Scand.* **13** (1959) 1474.